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IcoMST
2017-08

Bao , Y & Ertbjerg , P 2017 , Oxidation increases myofibrillar protein net charge . in D Troy , C McDonnell , L Hinds & J Kerry (eds) , Proceedings of 63rd International Congress of Meat Science and Technology : Nurturing locally, growing globally . IcoMST , pp. 834-835 , 63rd International Congress of Meat Science and Technology , Cork , Ireland , 13/08/2017 . https://doi.org/10.3920/978-90

http://hdl.handle.net/10138/232702
https://doi.org/10.3920/978-90-8686-860-5

publishedVersion

Oxidation increases myofibrillar protein net charge

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Abstract – In order to study the effect of oxidation on the charges of myofibrillar proteins, extracted myofibrils were incubated with different concentrations of the oxidant NaClO (0, 1, 5, and 10 mM). Isoelectric focusing showed that the isoelectric point (pI) of oxidized proteins were lower compared to non-oxidized ones. The lower pI values of oxidized proteins indicated that oxidation increased the overall net negative charges of proteins.

Key Words – carbonyls, isoelectric point, isoelectric focusing gel, protein functionality

I. INTRODUCTION

The biophysical properties of myofibrils are greatly affected by ionized fixed-charge groups attached to the backbones of macromolecules as those constituting the myosin and actin filaments. Many of the amino acid sidechains (such as Asp, Glu, His, Cys, Lys, Arg) can pick up or lose protons, and thereby alter the charge on the side chain. In meat, protein oxidation has been reported to modify some amino acid sidechain groups [1], which may have an effect on the net charges of proteins. Protein net charges can be evaluated by isoelectric focusing which separates proteins based on their isoelectric point (pI). By definition, pI is the pH at which the protein molecule has a net charge of zero. This paper aims to examine the effect of oxidation on the net charges of myofibrillar proteins by isoelectric focusing gel analysis.

II. MATERIALS AND METHODS

Porcine *longissimus thoracis et lumborum* (LTL) muscle was used in the present study. Myofibrils were extracted in MES buffer (100 mM KCl, 50 mM MES (2-(N-Morpholino) ethanesulfonic acid hydrate, 4-Morpholineethanesulfonic acid) hydrate, 2mM $MgCl_2$, 2 mM EGTA (ethylene glycol tetraacetic acid), pH 5.5) following oxidation with the oxidant NaClO at different concentrations (0, 1, 5, and 10 mM NaClO). Carbonyl content was determined to measure protein oxidation as described by Soglia et al [2]. The oxidized proteins were extracted with extraction solution (8 M urea, 2 M thiourea, 1% CHAPS) and separated by isoelectric focusing gel. After separation, gels were stained with Coomassie Brilliant Blue R-250 and western blot towards myosin heavy chain was performed.

III. RESULTS AND DISCUSSION

Myofibrillar proteins were extracted following oxidation by a strong chemical oxidant (NaClO) and separated according to their pI values (Fig. 1). The isoelectric focusing gel showed that the pI of the proteins were generally shifted to a slightly more acidic area (box 1 – 4, Fig. 1A). Box 1 corresponds to a pI around 6.6, while the intact myosin heavy chain in porcine skeletal muscle has a theoretical pI around 5.6 (from UniProtKB). The proteins/peptides in Box 1 and 2 were recognized by the myosin heavy chain antibody (Fig. 1B) suggesting that they contain fragments of myosin heavy chain. The protein bands had clearly migrated to a lower position in the 10 mM NaClO group, indicating that the pI value of myosin heavy chain had shifted towards the acidic area. Box 3 (pI around 5.4) and box 4 (pI around 5.2) were unidentified in the present study (Fig. 1A).

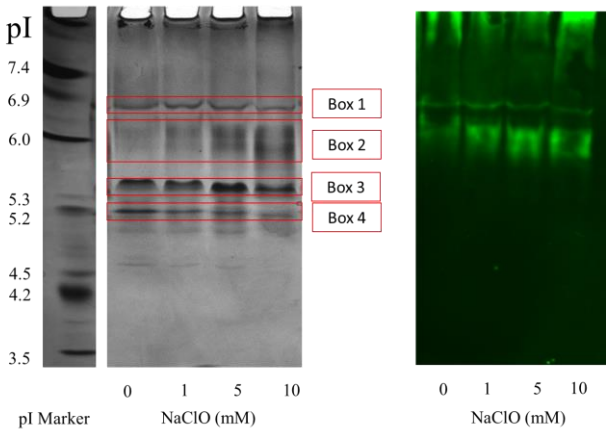

Fig. 1. Representative isoelectric focusing gel of porcine myofibrillar proteins oxidized with various amounts of NaClO and stained with Coomassie Brilliant Blue R-250 (A) or antibody towards myosin heavy chain (B).

In agreement to our observed lower pI upon oxidation, it was reported that some proteins involved in the oxidative stress response in human myoblasts were shifted to a more acidic pI upon oxidative stress [3]. Oxidation-induced modifications on amino acid side chain may alter the protein charges and thereby change the pI. As an example, as reviewed by Estévez [1], the ε-amino group in the lysine residue loose the positive charge when lysine forms a carbonyl. The loss of a positive charge will increase the overall net negative charge whereby the pI of protein becomes lower. In the present study, the carbonyl content increased with increasing oxidant concentration (data not shown), and at the same time the pI of oxidized proteins were shifted to a more acidic area suggesting that protein carbonylation lead to an overall increase in net negative charges of the proteins.

Protein net charges have been linked to protein functionality in meat. As an example, increased net filament charges have been hypothesized to induce swelling of myofibrils due to filament repulsion and/or greater osmotic pressure, and thereby improve water-holding capacity. Oxidation-induced increase in the net negative charge may thus be a key factor to understand the functionality of oxidized proteins.

IV. CONCLUSION

Myofibrillar proteins shifted to a more acidic pI upon the addition of the oxidant NaClO, indicating an increase in the net negative charge of the affected proteins. Alterations in pI of myofibrillar proteins may provide a novel perspective to understand oxidation-induced meat quality changes.

ACKNOWLEDGEMENTS

The authors thank the China Scholarship Council for the financial support.

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